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The inhibitory effects of ranitidine and cimetidine on propranolol elimination by the rat isolated perfused liver

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The effect of low (50 μg) and high (1 mg) doses of the histamine H_2-receptor antagonists cimetidine and ranitidine on the first pass extraction of propranolol was studied in the rat isolated perfused liver. Both low and high dose cimetidine increased the area under the perfusate propranolol concentration time curve (AUC) 4 to 5-fold. Although low dose ranitidine did not alter propranolol AUC, high dose ranitidine increased it to the same extent as cimetidine. These results indicate that ranitidine has a clear propensity for microsomal inhibition, but one which is unlikely to be manifest at therapeutic dosage.

The H_2-antagonist, cimetidine, has been shown to retard the hepatic elimination of both low and high clearance drugs (Serlin et al 1979; Klotz & Reimann 1980; Feely et al 1981, 1982). Ranitidine is largely free of this effect (Henry et al 1980), and this has been attributed to its different chemical nucleus. However, both H_2-antagonists have been shown to bind to and inhibit microsomal cytochrome P450 in-vitro, although binding and inhibition were both much weaker with ranitidine than with cimetidine (Rendic et al 1982). The question therefore arises whether this apparent lack of effect of ranitidine in-vivo is simply related to dose, rather than to a fundamentally different chemical interaction with cytochrome P450. This study therefore examines the effect of ranitidine on the elimination of the high clearance drug propranolol in the rat isolated perfused liver at high and low dose, and compares its effect with that of cimetidine.

Methods

Experimental preparation. Non-fasting, male Sprague-Dawley rats, 195–260 g, were anaesthetized by ether, and livers removed by standard techniques (Gollan et al 1981). After cannulation of the bile duct, portal vein and inferior vena cava the liver was transferred to a humidified glass chamber and connected to the perfusate circuit. The 100 ml circuit was housed in a thermostatically-controlled cabinet at 37 °C. The perfusate was recirculated by a peristaltic pump (LKB Model 2115 Multipurpex) at a constant flow (16 ml min^{-1}) from the perfusate reservoir, through a millipore filter, silastic membrane oxygenator, bubble trap and pressure manometer to the portal vein inflow cannula.

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The perfusate consisted of 10% v/v washed human red cells, 1% w/v bovine serum albumin (Commonwealth Serum Laboratories, Melbourne, Australia), and 0·1% w/v glucose in a standard electrolyte solution (Bartosek et al 1973) equilibrated with 95% oxygen and 5% carbon dioxide at pH 7·4. Bile flow was maintained by a constant background infusion of sodium taurocholate (30 μmol h^{-1}) (Calbiochem, San Diego, California, USA) into the perfusate reservoir.

The principal indices of liver viability were steady oxygen consumption (1·5–2·0 μmol O_2 g^{-1} liver min^{-1}), sustained bile production (0·5–1·0 ml h^{-1}) stable perfusate transaminase levels (6–50 IU $litre^{-1}$) and normal appearance on light microscopy (Jones et al 1984).

Experimental design. Propranolol was administered via the pre-systemic route in a total of 20 rat livers. In each experiment an initial 200 μg bolus dose of propranolol was administered directly into the portal vein. Perfusate (1·5 ml) was sampled from the reservoir at 0, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45 and 60 min for estimation of propranolol. An equal volume of perfusate was added to the reservoir to replace that removed by sampling. At the end of 60 min fresh perfusate was connected to the liver, and 5 min allowed for equilibration. In test experiments, H_2-antagonist was added to the reservoir at the beginning of the equilibration period. A second 200 μg bolus dose of propranolol was then administered via the same route, and perfusate sampled from the reservoir as for the first 60 min period. Further perfusate samples were taken predose and at 60 and 120 min after the 1st and 2nd dose of propranolol for pO_2 and transaminase estimations (5 ml). A larger volume (2 ml) was taken predose and at 2, 30 and 60 min after the 2nd dose of propranolol for estimation of H_2-antagonist concentration. This design enabled measurement of propranolol elimination without and with concomitant H_2-antagonist in the same liver.

In 4 of the livers (as controls), no H_2-antagonist was added to the reservoir at the start of the 5 min equilibration before the second bolus dose of propranolol. In the test groups, a high (1 mg) dose of cimetidine was added to the reservoir before the second propranolol dose in 4 livers, a high (1 mg) ranitidine dose was used in another 4 livers, a low (50 μg) dose of cimetidine was used in a further 4 livers, whilst in the remaining 4 livers a low (50 μg) ranitidine dose was used.

In four further rat livers, the same dose of propranolol was added directly to the reservoir, thereby simulating systemic dosage. Perfusate samples were taken as above. The results from this group allowed calculation of extraction ratio (see below).

Drug assays. After separation of red cells, an aliquot (0·5 ml) of perfusate was assayed by high pressure liquid chromatography for propranolol (minimum sensitivity 1 ng ml^{-1}) (Mihaly et al 1982a). The with-in day coefficient of variation at high (200 ng ml^{-1}) and low (5 ng ml^{-1}) propranolol concentrations was 1·7% (n = 6) and 11·6% (n = 6) respectively. In the selected samples cimetidine (minimum sensitivity 25 ng ml^{-1}) (Mihaly et al 1982b) and ranitidine (minimum sensitivity 5 ng ml^{-1}) (Mihaly et al 1980) were also measured in separate 0·5 ml aliquots by HPLC.

Statistics and calculations. Data are presented as mean ± s.d. The area under the perfusate concentration-time curve from 0–60 min (AUC_{0-60}) was calculated by the trapezoidal method (Gibaldi & Perrier 1982). Extraction ratio (E) was calculated by one minus the ratio of AUC_{0-60} after pre-systemic dosage to AUC_{0-60} after systemic dosage. Differences in AUC_{0-60} between the first hour and the second hour were statistically analysed by the Student's paired *t*-test. In all comparisons, a *P* value of less than 0·05 was taken as the minimum level of significance.

Results

In the control experiments, pre-systemic administration of 200 µg of propranolol directly into the portal vein resulted in an AUC_{0-60} of 239·5 ± 228·8 ng min ml^{-1} (Table 1). A second pre-systemic dose gave an AUC_{0-60} of 252·9 ± 243·4 ng min ml^{-1}, which was not significantly different ($P > 0·70$).

Addition of 1 mg cimetidine to the reservoir before the second dose of propranolol resulted in a 5·3 fold increase in AUC_{0-60} ($P < 0·01$). Similar addition of 1 mg ranitidine resulted in a 4·9 fold increase in AUC_{0-60} ($P < 0·05$) (Table 1).

After 50 µg of cimetidine, there was again a substantial increase (4-fold) in propranolol ($P < 0·05$) (Table 1). Ranitidine 50 µg did not change the AUC_{0-60} significantly ($P > 0·50$, Table 1).

After systemic dosage into the reservoir, a 200 µg bolus dose of propranolol was rapidly eliminated with a half-life of 2·55 ± 0·60 min, resulting in an AUC_{0-60} of 8530 ± 1746 ng min ml^{-1}. Comparison of AUC_{0-60} from presystemic dosage with that from systemic dosage showed that the extraction ratio (E) was high in all preparations and varied from 0·930 (liver 4, Table 1) to 0·998 (liver 9). However, this small variation in E represents a very large variation in available drug that escapes first pass extraction (1-E) by the liver. Since AUC_{0-60} of presystemically administered drug is directly proportional to (1-E), considerable variability between livers in AUC_{0-60} of presystemically administered drug is to be expected (Table 1). Each liver, however, acted as its own control, and data were analysed by paired *t*-testing.

Table 1. Effect of H_2-antagonists on area under propranolol concentration versus time profile (AUC_{0-60}).

	Liver	AUC_{0-60} min 1st h	AUC_{0-60} min 2nd h†
Control (no H_2-antagonist)	1	72·4	53·1
	2	127·6	240·1
	3	181·7	119·2
	4	576·1	599·0
	Mean ± s.d.	239·5 ± 228·8	252·9 ± 243·4
1 mg cimetidine	5	22·2	261·6
	6	24·2	516·4
	7	110·7	343·4
	8	164·6	584·9
	Mean ± s.d.	80·4 ± 69·7	426·6 ± 149·7**
1 mg ranitidine	9	18·3	110·9
	10	34·4	591·1
	11	35·2	158·4
	12	143·9	287·0
	Mean ± s.d.	58·0 ± 57·8	286·9 ± 216·0*
50 µg cimetidine	13	25·5	167·0
	14	50·6	421·4
	15	67·7	94·9
	16	92·7	260·9
	Mean ± s.d.	59·1 ± 28·3	236·1 ± 141·0*
50 µg ranitidine	17	52·3	95·9
	18	149·0	195·5
	19	168·0	222·7
	20	239·4	164·1
	Mean ± s.d.	152·2 ± 77·1	169·4 ± 54·6

† Dose of H_2-antagonist added to reservoir during 5 min equilibration period between 1st and 2nd h.
* Significantly greater than 1st h, $P < 0·05$.
** Significantly greater than 1st h, $P < 0·01$.

Following a dose of 1 mg of cimetidine, perfusate concentrations of cimetidine declined monoexponentially to 7·03 ± 0·83, 3·71 ± 0·30 and 2·35 ± 0·20 µg ml^{-1} at 2, 30 and 60 min respectively. A similar profile was observed after 50 µg of cimetidine with perfusate concentrations of 252 ± 95, 165 ± 98 and 48 ± 32 ng ml^{-1}. Ranitidine perfusate concentrations at 2, 30 and 60 min were 5·76 ± 0·19, 2·60 ± 0·28 and 1·38 ± 0·72 µg ml^{-1} after 1 mg dose and 353 ± 35, 134 ± 12 and 60 ± 12 ng ml^{-1} after 50 µg. This confirmed that perfusate H_2-receptor antagonist levels were sustained and remained at (low dose experiments) or above (high dose experiments) the levels seen with clinical usage (Mihaly et al 1984).

Discussion

Cimetidine has been shown to inhibit the hepatic elimination of low clearance drugs such as warfarin (Serlin et al 1979) and diazepam (Klotz & Reimann 1980) and high clearance drugs such as propranolol (Feely et al 1981) and lignocaine (Feely et al 1982). The mechanism of this interaction between cimetidine and low clearance drugs is due to inhibition of microsomal cytochrome P450 enzyme activity i.e. inhibition of intrinsic clearance. The interaction is apparent irrespec-

tive of the route of administration of the low clearance drug.

With high clearance drugs given *systemically* (Feely et al 1981, 1982) there may be no discernible decrease in systemic clearance due to cimetidine, because flow rather than intrinsic clearance is the rate limiting factor with this route of administration. In contrast, following *pre-systemic* (i.e. oral) administration of high clearance drugs, the decrease in intrinsic clearance due to cimetidine will be reflected directly in higher systemic blood levels of drug. Thus it is not surprising that the elimination of propranolol given orally is retarded by cimetidine treatment (Feely et al 1981).

In the present study, portal vein, i.e. pre-systemic, administration of propranolol in the isolated perfused rat liver allowed direct investigation of the influence of the two H_2-receptor antagonists on the intrinsic clearance of the high clearance drug propranolol. In contrast to previous studies both 'therapeutic' and 'supra-therapeutic' doses of cimetidine and ranitidine were examined. As expected, at both high and low cimetidine doses, hepatic intrinsic clearance of propranolol was reduced substantially, as reflected by the large increases in propranolol AUC's compared with control (Table 1). Also expected was the lack of effect of the low dose of ranitidine on propranolol intrinsic clearance, when perfusate ranitidine concentrations were comparable to plasma ranitidine concentrations seen during ranitidine treatment in patients (Mihaly et al 1984).

However, marked inhibition was evident at the higher ranitidine dose (Table 1). Although this finding might not be of immediate clinical importance, it does provide information on the mechanism of inhibition of drug metabolism by H_2-receptor antagonists. The lack of inhibition by therapeutic doses of ranitidine in previous studies (Henry et al 1980) had reinforced the belief that a requirement for inhibition of drug metabolism by H_2-antagonists was the presence of the imidazole nucleus on which cimetidine is based. The profound inhibition of propranolol metabolism by the higher dose of ranitidine in the present study, equal to that produced by cimetidine, makes this hypothesis untenable. Both H_2-receptor antagonists probably inhibit by similar mechanisms, with cimetidine simply being a more potent inhibitor.

This interpretation is in accord with in-vitro binding studies (Rendic et al 1982) which have shown that ranitidine does form a ligand complex with cytochrome P450. However, the affinity of ranitidine for cytochrome P450 is one tenth that of cimetidine, and the two H_2-receptor antagonists appear to interact with different forms of cytochrome P450. The inhibition of 7-ethoxycoumarin dealkylation by ranitidine was less than half that of a similar dose of cimetidine (Rendic et al 1982).

The present study clearly shows that ranitidine at high dose has an equal propensity for microsomal inhibition, but the lack of effect at the lower 'therapeutic' dose makes it unlikely that a clinically important interaction will occur.

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